Total and pneumococcal-specific IgA levels did not change after initiation of HAART. No change was detected in the levels of pneumococcal-specific IgA when expressed as a percentage of total IgA. No significant correlation was found between BAL IL-6 and IgA concentration or BAL percentage lymphocytes and IgA concentrations. **Conclusion:** HAART is not associated with significant changes in total or pneumococcal-specific IgA concentrations in the distal respiratory tract. We speculate that this reflects the more dominant role of IgG in protection of the alveolar space against bacterial pathogens.

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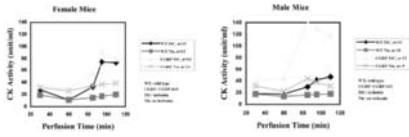
BIOINFORMATIC CLASS DISCOVERY OF POTENTIAL BIOMARKERS IN VENTILATOR-ASSOCIATED LUNG INJURY. S.-F. Ma,[1] D.N. Grigoryev,[2] R.B. Easley,[3] S.Q. Ye,[1] B.A. Simon,[3] J.G N. Garcia,[1] [1]Critical Care, The University of Chicago, Chicago, IL; [2]Clinical Immunology and [3]Anesthesiology and Critical Care, Johns Hopkins University, Baltimore, MD.
Rationale: The unilateral (one lung injured and the contralateral lung intact) model of ventilator-associated lung injury (VALI) allows us to utilize uninjured lung as the systemic VALI effects indicator. We speculated that regulation of genes in uninjured lung will be attributed to blood or lymph carrying effectors produced by an injured lung and the analysis of upstream bioprocesses governed by affected genes will lead to the effectors' discovery. **Methods:** Dogs (n = 4) were intubated, left lung lavaged, and then both lungs were either independently ventilated (total Vt = 15 mL/kg) for 6 hours (injured and uninjured) or immediately harvested (control). Lung mRNA (n = 16 for all three groups) was hybridized to HG_U133A and analyzed by SAM using interspecies probe adjustment. Genes with the lowest false discovery rate (q = 0.124%) that imposed fold change (FC) range from −3.52 to −1.26 and 1.22 to 6.96 with corresponding FC averages −1.59 and 2.01 were considered affected by VALI. Gene ontology filtering for receptor activity term was conducted by MAPPFinder. **Results:** Our analyses revealed 22 receptor-related genes, of which 7 were growth factor receptors including EGFR (FC = −1.54), FGFR1 (FC = −1.59), FGFR2 (FC = −1.56), and PDGFR (FC = −1.30). The overall down-regulation of these receptors was concordant with decreased expression of their corresponding ligands in injured lung including EGF (FC = −1.39), FGF2 (FC = −1.39), PDGFA (FC = −1.41), and PDGFB (FC = −2.00). Fibroblast (Z = 6.17) and epidermal (Z = 4.49) growth factor receptor activity were the first and the third significantly (Z > 1.96) affected pathways. **Conclusions:** Our approaches effectively identify a class of potential biomarkers in VALI. Further investigation of this study may elucidate systemic VALI effects and facilitate the development of new diagnostic tools.



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MYOCARDIAL ISCHEMIA CAUSES HIGHER CREATINE KINASE RELEASE IN CALCITONIN GENE–RELATED PEPTIDE KNOCKOUT MALE MOUSE HEARTS. H. Ma, R Huang, A. Carve, I. Shah, S.C. Supowit,* D.J. DiPette,* G.S. Abela, Department of Medicine, Michigan State University, East Lansing, MI; *Texas A & M University, Scott and White Hospital, Temple, TX.
Background: Calcitonin gene–related peptide (CGRP) influences vasoregulatory activities. We determined the gender-specific effects of CGRP knockout (KO) on creatine kinase (CK) activity following ischemia. **Methods:** Ninety-six mice were studied in a Langendorff preparation using 50 mm Hg perfusion pressure. Myocardial ischemia was induced by stopping flow to the coronary arteries for 20 minutes. The perfusion buffer was collected from a small chamber that housed the heart. Perfusion buffer was collected over 2 hours and CK activity was measured. **Results:** CK activity was significantly greater in CGRP-KO mouse hearts compared to wild-type (see graphs). Male CGRP-KO hearts released 1.5 times more CK than female CGRP-KO hearts 15 minutes after ischemia (130.4 vs 90.2 unit/mL, $p < .003$). **Conclusions:** CGRP contributes significantly to CK release during ischemia. This effect is more prominent in the male compared to the female mouse heart.

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OXIDIZED PHOSPHOLIPIDS MEDIATE INTERACTION BETWEEN ADHERENCE JUNCTION AND FOCAL ADHESION PROTEINS. I. Malyukova,[1] A. A. Birukova,[1] A. Rios,[2] K.G. Birukov,[1] [1] Department of Medicine, The University of Chicago, Chicago, IL; [2] Department of Medicine, Johns Hopkins University, Baltimore, MD.
Introduction: Oxidized phospholipids appear in the pulmonary circulation as a result of acute lung injury or inflammation. We have previously shown that oxidized phospholipids exhibit barrier-protective effects on pulmonary endothelial cell (EC) monolayers. However, effects of oxidized phospholipids on EC focal adhesion (FA) and adherence junction (AJ) remodeling have not been yet fully explored. **Goal:** To study molecular mechanisms of adherens junction and focal adhesion remodeling mediated by oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (OxPAPC). **Methods:** All experiments were performed on human pulmonary artery endothelial cells (HPAEC). Intracellular protein localization was analyzed by immunocytochemistry. Subcellular localization of the proteins of interest was determined using subcellular proteome extraction kit. Protein phosphorylation profile was assessed by Western blot analysis. Protein-protein interactions were analyzed by coimmunoprecipitation assays. **Results:** Enhancement of EC barrier in response to OxPAPC was accompanied by dynamic remodeling of focal adhesions and adherence junctions. Immunofluorescent analysis of OxPAPC-stimulated EC revealed dramatic translocation and peripheral and enhanced peripheral staining for AJ proteins beta-catenin and VE-cadherin. In addition, OxPAPC treatment increased tyrosine phosphorylation of FA proteins FAK at Tyr-576 and paxillin at Tyr-118, which was associated with peripheral redistribution of FA and AJ complexes. Furthermore, subcellular fractionation analysis showed increase of VE-cadherin, beta-catenin, and GIT2 in membrane fraction after OxPAPC challenge. Remarkably, coimmunoprecipitation studies indicated increased interaction of paxillin with FA components FAK, vinculin and GIT2, and AJ protein beta-catenin upon HPAEC stimulation with OxPAPC. Complementary experiments with immunoprecipitation beta-catenin followed by probing for paxillin confirmed these results. **Conclusions:** The results of these studies characterize OxPAPC-induced focal adhesion remodeling and determine for the first time the specific interactions between focal adhesion and adherens junction protein complexes in endothelial barrier-protective responses to OxPAPC

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***BACILLUS ANTHRACIS* SPORES STIMULATE CYTOKINE AND CHEMOKINE INNATE IMMUNE RESPONSES IN HUMAN ALVEOLAR MACROPHAGES THROUGH MULTIPLE MAPK PATHWAYS.** K. Chakrabarty, W. Wu, J.L. Booth, E.S. Duggan, K.M. Coggeshall, J.P Metcalf, Pulmonary and Critical Care Division, Department of Medicine, University of Oklahoma Health Sciences Center, and the Program in Immunobiology and Cancer, Oklahoma Medical Research Foundation, Oklahoma City, OK.
Contact with the human alveolar macrophage plays a key role in the innate immune response to *Bacillus anthracis* spores. Because there is a significant delay between the initial contact of the spore with the host and clinical evidence of disease, there appears to be temporary containment of the pathogen by the innate immune system. Therefore, the early macrophage response to anthrax exposure is important in understanding the pathogenesis of this disease. We examined the initial events after exposure of human alveolar macrophages obtained by bronchoscopy to *Bacillus anthracis (Sterne)* spores. Spores were rapidly internalized as determined by confocal microscopy. Spore exposure also rapidly activated the MAPK signaling pathways ERK, JNK, and P38. This was followed by transcriptional activation of cytokine and primarily monocyte chemokine genes as determined by RNase protection assays. Transcriptional induction was reflected at the translational level as IL-1α and β, IL-6, and TNF-α cytokine protein levels were markedly elevated as determined by ELISA. Induction of IL-6 and TNF-α, and to a lesser extent IL-1α and -β, was partially inhibited by blockade of individual mitogen-activated protein kinases, while complete inhibition of cytokine induction was achieved when multiple signaling pathway inhibitors were used. Taken together, these data clearly show activation of the innate immune system in human alveolar macrophages by *Bacillus anthracis* spores. The data also show that multiple signaling pathways are involved in this cytokine response.

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A REEVALUATION OF EVANS BLUE DYE AS A MARKER OF ALBUMIN CLEARANCE IN MOUSE MODELS OF ACUTE LUNG INJURY. S. Sammani, J. Moitra, J.G.N. Garcia, Department of Medicine, The University of Chicago, Chicago, IL.
Background: Quantifying the amount of albumin conjugated to Evans Blue dye (Alb-EB) fluxing across organ barriers is a popular technique to measure intactness of the physical barrier in rodent models of a variety of diseases. We have reevaluated this technique in terms of the correction factors required in a spectrophotometric assay. **Methods and Results:** Eight- to 10-week-old C57BL6/J mice received either pH-neutral water (controls) or LPS (treatment) by intratracheal instillation, and acute lung injury was allowed to develop for 24 hours. Both control and treatment mice were further injected with Alb-EB via the jugular vein, at doses of either 20 or 30 mg/kg body weight, at 30, 60, 120, or 180 minutes before termination of the LPS treatment (24 hours total). At the end of exposure, formamide extracts of lungs were prepared, and the centrifuged supernatants were measured at 620 and 740 nm in a spectrophotometer. Lungs from control mice that were not injected with Alb-EB were similarly extracted and measured. The linear regression equation between absorbances at 740 nm (X) and 620 nm (Y) in control lung extracts of animals that did not get Alb-EB was considered to be the tissue-specific correction factor. The observed absorbances of the control and treatment samples at 620 and 740 nm were then normalized using this factor. This tissue-specific correction resulted in control samples read as positive integers, as opposed to negative integers when using a serum correction factor commonly used in the literature. We also determined that adjusting the duration of the conjugated dye in circulation is critical for maximizing the signal-to-noise ratio. **Conclusion:** The Evans Blue dye extravasation method to quantify barrier dysfunction can be improved in terms of repeatability and sensitivity by using tissue-specific correction factors and maximized signal-to-noise ratios, respectively.
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NOX4, A HOMOLOGUE OF NOX2, REGULATES HYPEROXIA-INDUCED REACTIVE OXYGEN SPECIES PRODUCTION AND ANGIOGENESIS IN HUMAN LUNG ENDOTHELIAL CELLS. S. Pendyala, I. Gorshkova, B. Gorshko, H. Donghong, R.K. Stern, P. Usatyuk, V. Natarajan, Department of Medicine, The University of Chicago, Chicago, IL.
Rationale: Nox4, a homologue of Nox2 ($gp91^{phox}$), is involved in ROS production and signal transduction in vascular cells. In human pulmonary artery endothelial cells (HPAECs), mRNA expression of Nox4 is several folds higher compared to Nox2 and exposure of cells to hyperoxia (95% O_2, 24 hours) resulted in up-regulation of Nox4 and $p22^{phox}$ but not Nox1 or Nox3. Nox4 siRNA partially reduced ROS formation and blocked cell motility and capillary tube formation in cells exposed to either normoxia or hyperoxia, suggesting a role for Nox4 in angiogenesis. **Methods/Results:** In HPAECs and human lung microvascular ECs, expression of Nox4 was several folds higher, as shown by real-time PCR, and exposure to hyperoxia (24 hours) up-regulated Nox4 mRNA as well as protein expression. The localization of Nox4 in HPAECs, as determined by immunofluorescence microscopy with Nox4 antibody, revealed that a majority of the native Nox4 protein was localized near the perinuclear region that stained positive for Golgi marker and a small fraction extended throughout the cytoplasm in internal membrane and vesicular structures. Exposure of cells to hyperoxia (3 hours) caused the Golgi to assume a rounded appearance from a saucer-shaped structure where in majority of Nox4 was colocalized. As hyperoxia-mediated cell motility was attenuated by Nox4 siRNA and was dependent on ROS production, we studied the role of Nox4 in capillary tube formation using matrigel assay. Exposure of HPAECs grown on matrigel to hyperoxia (24 hours) increased the number of capillary tubes compared to normoxia and Nox4 siRNA attenuated the capillary tube formation. **Conclusion:** Nox4 participates in ROS production and acts as a signaling protein that plays a pivotal role in regulating key EC functions such as migration and capillary tube formation.
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5-HYDROXYTRYPTAMINE 4 RECEPTOR IN THE ENDOTHELIAL CELLS. I. Profirovic, I. Vardya, T. Voyno-Yasenetskaya, Department of Pharmacology, University of Illinois at Chicago, Chicago, IL.
Serotonin (5-hydroxytryptamine [5-HT]) is an important neurotransmitter that regulates multiple events in the central nervous system (CNS). We have recently demonstrated that 5-HT4 receptor couples to G13 protein to induce RhoA-dependent gene transcription, neurite retraction, and neuronal cell rounding (Ponimaskin et al, 2002). Although multiple

studies were focused on the function of the 5-HT4 receptor in the CNS, none of the studies showed its expression and function in the endothelial cells. In the present study, we provide evidence for the first time that 5-HT4 receptor is expressed in the human umbilical vein endothelial cells (HUVECs). We demonstrate the transcription of 5-HT4 mRNA in the HUVECs using reverse transcription polimerase chain reaction. Additionally, we show 5-HT4 receptor expression in HUVECs by immunoblotting and immunofluorescent analysis with 5-HT4 specific antibody. Importantly, we determine that overexpression of 5-HT4 receptor leads to a pronounced cell rounding and intercellular gap formation in HUVECs. We are currently investigating the mechanism underlying 5-HT4 receptor-induced actin cytoskeleton changes in the endothelial cells. These data suggest that by activating 5-HT4 receptor, serotonin could be involved in regulation of actin cytoskeleton dynamics in the endothelial cells that may affect the endothelial barrier integrity.

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OVEREXPRESSION OF ANTIOXIDANT ENZYMES IN LUNG EPITHELIAL CELLS PREVENTS DAMAGE FROM AIRBORNE PARTICULATE MATTER–INDUCED OXIDANT INJURY. S. Soberanes,[1] V. Panduri,[1] H. Wang,[1] G. Mutlu,[1] G.R. Budinger,[1] D.W. Kamp,[1,2]
[1]Department of Medicine, Divisions of Pulmonary and Critical Care Medicine, Northwestern University Feinberg School of Medicine; [2]Veterans Administration Chicago Health Care System: Lakeside Division, Chicago, IL.
Elevated levels of air pollution particles are associated with increased morbidity and mortality from acute and chronic cardiopulmonary injury. One mechanism underlying these effects involves oxidative damage to lung epithelial cells. We previously showed that Düsseldorf particulate matter (DPM) causes alveolar epithelial cell DNA damage and apoptosis by a mitochondria-regulated death pathway. In this work, we used several different types of well-characterized particulates to determine whether mitochondria-derived reactive oxygen species (ROS) are the primary cause of apoptosis. Washington particulate matter (WPM), residual oil fly ash (ROFA), and DPM each increased ROS production (ASSAY HERE) and apoptosis (DNA fragmentation) as compared to inert particulates such as desert dust (DD) and Mount St Helen volcanic dust (MSH) (Table 1). Notably, WPM, ROFA, and DPM did not induce ROS production or apoptosis in ;gr0-A549 cells, which are incapable of mitochondrial ROS production. We also found that overexpression of MnSOD or CuZnSOD using adenoviral expression vectors blocks DPM-induced A549 cell ROS production and apoptosis as compared to null/sham adenoviral controls. We conclude that a diverse group of toxic airborne particulates, unlike inert particulates, induce mitochondria-derived ROS production and lung epithelial cell apoptosis. We propose that strategies aimed at reducing mitochondrial-derived ROS levels will protect the lung epithelium exposed to airborne particulate matter.

TABLE 1 Elevated Levels of ROS in Airborne Particulate Matter Increases A549 Cell Apoptosis

	DPM	*WPM*	*ROFA*	*DD*	*MSH*
ROS (% over control)	301.3 ± 20.0*	319.3 ± 8.0*	146.1 ± 15*	8.38 ± 2.1	9.77 ± 1.7
DNA frag. (% over control)	70.4 ± 5.9*	37.4 ± 5.3*	58.8 ± 9.1*	0.98 ± 1.1	0.98 ± 0.25

Mean ± SEM ($n = 3$). *$p < .05$ vs untreated cells.

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EXPANSION OF SUBPOPULATIONS OF *BACTEROIDES* THAT HYPERPRODUCE BETA-LACTAMASE PREVENTS CEFTRIAXONE-INDUCED DISRUPTION OF THE HUMAN INTESTINAL MICROFLORA IN A CONTINUOUS-FLOW CULTURE MODEL. U. Stiefel,[1,2] N.J. Pultz,[2] C.J. Donskey,[1,2] [1]Case Western Reserve University, [2]VA Medical Center, Cleveland, OH.
Background: The indigenous intestinal microflora may generate beta-lactamase activity in response to beta-lactam antibiotic selective pressure. We hypothesized that expansion of subpopulations of *Bacteroides fragilis* group organisms that hyperproduce cephalosporinases could prevent disruption of the indigenous microflora in the face of ceftriaxone selective pressure. **Methods:** In vitro mixing studies were performed to determine the ability of *Bacteroides fragilis* TAL3636 (BF3636), a hyperproducer of a broad-spectrum beta-lactamase, to raise the minimum bactericidal concentration (MBC) of susceptible organisms to ceftriaxone. Continuous-flow (CF) cultures of human colonic microflora were established with or without the addition of BF3636 and increasing concentrations of ceftriaxone were added to the inflowing media. Beta-lactamase activity, ceftriaxone concentrations, and densities of bacterial populations were monitored. A mouse model was also utilized to evaluate the ability of BF 3636 to preserve colonization resistance in vivo. Concomitant with injections of ceftriaxone, mice received orogastric inoculation of CF culture containing BF3636, BF3636 culture alone, or normal saline (control group). Mice subsequently received a culture of vancomycin-resistant enterococcus (VRE) by orogastric gavage. Stool samples were collected on succeeding days and plated for the presence of VRE and resistant *Bacteroides* species. **Results:** In mixing studies, BF3636 raised the MBC of a ceftriaxone-susceptible *Escherichia coli* strain from < 0.25 to 64, whereas organisms that did not produce beta-lactamase did not ($p < .001$). The CF culture containing intestinal microflora was not affected when 8 µg/mL of ceftriaxone was added to the inflowing media, but 100 µg/mL resulted in disruption of the microflora and detection of ceftriaxone in the culture. In the CF culture containing BF3636, the population of *Bacteroides* species with high-level resistance to cephalosporins expanded over time and beta-lactamase activity increased; infusion of 100 µg/mL of ceftriaxone did not disrupt the indigenous microflora and ceftriaxone levels remained undetectable. In vivo, ceftriaxone-treated mice that had been orally inoculated with CF culture containing BF3636 did not develop intestinal overgrowth with VRE despite exposure; this was in contrast to ceftriaxone-treated mice that had received an oral inoculation with saline alone. **Conclusion:** Expansion of subpopulations of *Bacteroides* species that hyperproduce cephalosporinases can inactivate high concentrations of ceftriaxone and preserve the indigenous intestinal microflora. CF culture containing beta-lactamase-producing *Bacteroides* species was able to preserve colonization resistance of ceftriaxone-treated mice against VRE.

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THE N-TERMINUS OF FOG-2 INTERACTS WITH METASTASIS-ASSOCIATED PROTEINS 1 AND 2 TO MEDIATE TRANSCRIPTIONAL REPRESSION DURING CARDIAC DEVELOPMENT. S.A. Samant, A.E. Roche, E.C. Svensson, Department of Medicine, The University of Chicago, Chicago, IL.
Mutations in several transcription factors that regulate cardiac development have recently been described to cause congenital heart disease in humans. A greater understanding of the molecular mechanisms that regulate heart formation may help identify other genes that when mutated will result in human congenital heart disease. FOG-2 is one such gene that encodes a transcriptional corepressor expressed in the developing heart. It is critical for proper cardiac morphogenesis as mice deficient in this factor die during midgestation of cardiac malformations. We have previously shown that FOG-2 physically interacts with GATA4 and attenuates GATA4's ability to activate cardiac specific gene expression. This repression is mediated by a domain of FOG-2 localized to its N-terminus, termed the FOG repression motif. To gain further insights into the molecular mechanism responsible for this repression, we took a biochemical approach to identify factors that interact with FOG repression motif. Using MALDI-TOF mass spectrometry, we identified seven proteins from rat neonatal cardiac nuclear extracts that copurified with a FOG-2-GST fusion protein. All of these proteins have been previously described to be subunits of a nucleosome-remodeling complex called the NuRD complex. To determine which of the NuRD subunits directly interact with the FOG repression motif, we used a series of in vitro binding assays. We found that MTA-1 and MTA-2 specifically bound to the N-terminus of FOG-2 but not to a mutant form of the N-terminus that is unable to mediate repression. In situ hybridization revealed that both MTA-1 and MTA-2 are expressed in the developing heart during mouse embryogenesis. Taken together, these results suggest that FOG-2 mediates transcriptional repression during cardiac development by the recruitment of the NuRD complex to GATA-dependent promoters leading to the remodeling of the local chromatin structure and the attenuation of gene expression.
This work was supported by NIH HL071063 and a grant from the Schweppe Foundation.

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THE ROLE OF PROTEIN PHOSPHATASE 2A IN THE REGULATION OF ENDOTHELIAL CELL CYTOSKELETON STRUCTURE. K. Tar,[1,2,#] C. Csortos,[1,2,#] I. Czikora,[2] G. Olah,[1] S-F. Ma,[1] R. Wadgaonkar,[1] P. Gergely,[2] J.G.N. Garcia,[1] A.D. Verin,[1*] [1]Department of Medicine, Division of the Biological Sciences, The University of Chicago, Chicago, IL; [2]Department of Medical Chemistry, Research Center for Molecular Medicine, University of Debrecen, Medical and Health Science Center, Debrecen, Hungary.
Our recently published data suggested the involvement of protein phosphatase 2A (PP2A) in endothelial cell (EC) barrier regulation (Tar et al, 2004). In order to further elucidate the role of PP2A in the regulation of EC cytoskeleton and permeability, PP2A catalytic (PP2Ac) and A regulatory (PP2Aa) subunits were cloned and human pulmonary arterial EC (HPAEC) were transfected with PP2A mammalian expression constructs or infected with PP2A recombinant adenoviruses. Immunostaining of PP2Ac or of PP2Aa+c overexpressing HPAEC indicated actin cytoskeleton rearrangement. PP2A overexpression hindered or at least dramatically reduced thrombin- or nocodazole-induced F-actin stress fiber formation and microtubule (MT) dissolution. Accordingly, it also attenuated thrombin- or nocodazole-induced decrease in transendothelial electrical resistance indicative of barrier protection. Inhibition of PP2A by okadaic acid abolished its effect on agonist-induced changes in EC cytoskeleton; this indicates a critical role of PP2A activity in EC cytoskeletal maintenance. The overexpression of PP2A significantly attenuated thrombin- or nocodazole-induced phosphorylation of HSP27 and tau, two cytoskeletal proteins, which potentially could be involved in agonist-induced cytoskeletal rearrangement and in the increase of permeability. PP2A-mediated dephosphorylation of HSP27 and tau correlated with PP2A-induced preservation of EC cytoskeleton and barrier maintenance. Collectively, our observations clearly demonstrate the crucial role of PP2A in EC barrier protection.
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AMBIENT PARTICULATE MATTER INDUCES MYOFIBROBLAST DIFFERENTIATION VIA MACROPHAGE-DEPENDENT TRANSFORMING GROWTH FACTOR β TYPE I (ALK5) SIGNALING. K. Thavarajah, R. Kalhan, A. Nair, M.C. Nlend, N. Wang, P.H.S. Sporn, Northwestern University, Chicago, IL.
Rationale: Increased levels of ambient particulate matter have been associated with increased pulmonary morbidity and mortality. To investigate if particulate matter induces airway remodeling, we studied the effects of particulate matter (< 10 µm in diameter) collected from Dusseldorf, Germany (DPM), on fibroblast to myofibroblast differentiation. **Methods:** Human fetal lung fibroblasts (IMR-90) were grown to subconfluence, serum-starved for 48 hours, and exposed to either TGF-β1 (2 ng/mL), DPM, or conditioned medium from human monocytic (THP-1) cells exposed to DPM. The role of TGF-β1 signaling was assessed by the addition of SB431542 (10 µM), a TGF-β type I (ALK5) receptor inhibitor. After 48 hours, cells were lysed and analyzed by immunoblot for α-smooth muscle actin (αSMA), a marker of myofibroblast differentiation. **Results:** Direct exposure of IMR-90 cells to DPM did not increase αSMA expression. However, conditioned medium of THP-1 cells exposed to DPM induced increased αSMA expression in fibroblasts. This increase was blocked by the ALK5 inhibitor SB431542. **Conclusions:** Ambient particulate matter triggers macrophage-dependent induction of myofibroblast differentiation via ALK5 receptor signaling. We speculate that particulate matter induces airway remodeling.

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REGULATION OF 4-HYDROXY-2-NONENAL–MEDIATED ENDOTHELIAL BARRIER FUNCTION BY FOCAL ADHESION AND ADHERENS JUNCTION PROTEINS. P.V. Usatyuk, V. Natarajan, School of Medicine, The University of Chicago, Chicago, IL.
4-Hydroxy-2-nonenal (4-HNE), a highly reactive aldehyde generated by peroxidation of membrane lipids, altered endothelial cell (EC) barrier function, resulting in vascular leakiness. Here we report that 4-HNE mediates EC barrier dysfunction by modulating focal adhesion and adherens junction proteins that affect cell-cell and cell-matrix interactions via integrins. Treatment of bovine lung microvascular endothelial cells (BLMVECs) with 4-HNE, in a dose-dependent manner, modulated cell-cell adhesion contacts, enhanced intracellular ROS formation, and increased permeability. Interestingly, 4-HNE did not alter